β-Glucan Ameliorates Gamma–Rays Induced Oxidative Injury in Male Swiss Albino Rats

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Abstract. 1, 3-β-D-Glucan is a natural polysaccharide derived from the cell walls of bakers yeast Saccharomyces cerevisiae with immunoenhancing and potent antioxidant effects. This study investigated the pathways through which β-glucan gavage treatment (50 mg/kg) exerts its effect on radiation-induced oxidative damage in male rats. β-glucan was given orally to male rats; 3 hours post γ-irradiation at dose 5 Gy, for 10 and 20 days post-irradiation level were assayed, being remarkable indicators in cell oxidative stress. Results pointed out that irradiation at 5 Gy significantly depressed all blood parameters, such as erythrocytes count (RBCs), hemoglobin content (Hb), hematocrit value (Hct), total leucocytes count and absolute lymphocytes and neutrophils counts. blood glutathione (GSH) level and conversely elevated level of serum ascorbyl radical (AsR), product of lipid peroxidation (MDA melanodialdehyde), triglycerides and cholesterol. Total leucocytes count and absolute lymphocytes and neutrophils counts, RBCs, Hb, Hct, blood GSH and serum MDA of irradiated animals receiving β-glucan administration were exhibited significant differences compared to the irradiated group. Marrow count and the percentage of viability and spleenocytes viability were also significantly decreased. β-glucan treatment accelerates recovery of cell damage induced by ionizing irradiation through its potential immune-enhancing activity and free radical scavenging ability that is partially mediated through stimulation of immunohaematological system thus could play a role in regulating irradiation complications.

Key Words: β-D-Glucan, γ-rays, oxidative injury, immunomodulatory, antioxidant properties

INTRODUCTION

Every year it is estimated that nearly 60% of all cancer patients receive radiation therapy, either alone or in conjunction with surgery or chemotherapy (Hogle, 2006). Irradiation ruptures adult tissue homeostasis, inducing radiation syndromes, described in hematopoietic tissue (for doses higher than 2 Gy, total body irradiation), in intestinal tissues (8 Gy), and in skin (12 Gy). It appears that a major mechanism of these syndromes is a rapid and massive cell death in stem and/or progenitor cell populations, which can follow either apoptotic or necrotic pathways (Harfouche and Martin, 2010). At clinically relevant doses of irradiation, bone marrow failure is the most commonly observed life-threatening problem. The observed effects are due to both a decrease in the number of hematopoietic stem cell progenitors and a reduction in self-renewal capacity of stem cells (Dainiak et al., 2003).

β-Glucans are a well-known biological response modifier that is widely distributed in nature. A variety of β-glucans have been isolated from different sources, e.g., from fungi, plants, and seaweed. The physicochemical properties of β-glucans vary depending on their structural features (Tsoni and Brown, 2008). Recent reports have highlighted the fact that β-glucans are effective in the treatment of cancer and infectious diseases and can be used in both modern medicine and traditional oriental therapeutic strategies. β-Glucans are also important dietary substances: they lower the plasma cholesterol level, enhance the hematopoietic response, and possess antitumor and immunomodulating properties (Torello et al., 2010; Borchers et al., 2004). Glucans enhance neutrophil recovery following sub-lethal irradiation (Gu et al., 2005). Ciprofloxacin (300 mg/Kg body wt.) and Echinacea (250 mg/Kg body wt) treatment accompanied by irradiation (2 Gy/week for 6 weeks) in female rats ameliorated the blood...
parameters, normalized K\(^+\) and ameliorated the GSH and advanced oxidation protein products which may suggest potential role in radiation therapy (Salama et al., 2007).

Rice et al. (2005) demonstrated that highly purified, water soluble glucans translocate from the gastrointestinal (GI) tract into the systemic circulation, persist in the plasma up to 24 h after a single oral administration and mediate its biological effects through binding with GI epithelial and gut-associated lymphoid tissue (GALT) cells and they modulate the expression of pattern recognition receptors in the GALT, increase IL-12 expression, and induce protection against infectious challenge.

β-Glucan administration was proposed as an alternative or complementary strategy to treat radiation-induced pathophysiology through stimulating residual hematopoietic residual cells and tripping free radicals accumulations after irradiation.

**MATERIALS AND METHODS**

**Animals**

Fifty six mature male Swiss albino rats weighing from 130 to 150g, were obtained from the National Center for Nuclear Researches, Inchas-Atomic Energy Authority- Egypt. All animal procedures were in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the Committee for Purpose of Supervision of Experiments on Animals (CPCSEA) and the National Institutes of Health protocol (NIH). Animals were allowed to acclimate in metal cages inside a well-ventilated room for 2 weeks prior to the experiment. They were maintained under standard laboratory conditions; temperature 23\(^\circ\)C, relative humidity 60-70% and a 12:12 h light/dark cycle and were fed a standard commercial pellet diet and water.

**Experimental design**

**Irradiation and β-glucan preparation**

Rats received β-glucans dosage (50 mg/kg/body wt), β 1,3 glucan is manufactured by The Vitamin Shoppe, North Bergen, NJ 07047) gavage for 10 and 20 days, 3 h after whole-body irradiation. It was dissolved in phosphate-buffered saline.

Whole body γ-irradiation was performed at the National Center for Radiation Research and Technology, Nasr City Cairo, Egypt using Cs-137 Gamma Cell 40 biological irradiator. Animals were irradiated at an acute single dose of 5Gy delivered at a dose rate of 0.87Gy/min.

Animals were divided into (i) Control group of 8 animals. (ii) sixteen animals were irradiated with gamma rays at a dose of 5Gy. (iii) sixteen animals were orally administered daily with β-glucan for 10 and 20 days (Sener et al., 2005). (iv) animals were irradiated with gamma rays at a dose of 5 Gy, 3 hours post irradiation, were orally administered daily with β-glucans (50 mg/kg). Control animals were sacrificed after 20 days, eight animals were sacrificed after 10 and 20 days in each experimental group.

**Collection of blood and tissue samples**

Two blood samples were immediately collected by heart puncture. The first sample was collected in heparinized tube (2.25µ heparin /5ml blood) for hematological assays. The second blood sample was centrifuged at 1000xg for 10 min, to collect the serum which was stored at -20°C until analyzed.

**Bone marrow suspension**

Femur bones were cleaned and chipped. The bone marrow was blow out into 5 ml saline solution, pooled and mixed by drawing and expelling it several times from the syringe without needle. Total number of bone marrow nucleated cells and their viability were counted using trypan blue on a hemocytometer (Goldberg et al., 1992).

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\text{viability}= \text{viable cells/total number X 100.}

**Spleen cells suspension**

After animal dissection, spleens were removed from rats in 0.1 M cold phosphate-buffered saline (PBS), and passed through a sterilized mesh to obtain single cell suspensions according to Yuan et al. (2006). Erythrocytes in the cell mixture were washed by the hypo-osmotic hemolysis rapidly. Cell viability of spleenocytes was evaluated by the trypan blue dye exclusion technique.
Hematological and biochemical assays

The first heparinized blood sample was used for RBC count, haemoglobin (Hb) estimation, haematocrit (Hct) determination and total leukocytes, absolute neutrophils and lymphocytes counts by automated blood counter (Coulter model T 450 x, Contronics Co., USA). Blood glutathione (GSH) content was measured according to Beutler et al. (1963) based on the determination of yellow color that develops when 5.5 dithiol-bis (2-nitrobenzoic acid) is added to sulphtydryl compounds. Serum malondialdehyde (MDA) was estimated according to Yoshioka et al. (1979), and was expressed as malondialdehyde equivalents. AsR was determined according to Nakagawa et al. (1997). Serum cholesterol and triglycerides were measured using kits according to Richmond (1973) and Fossati and Prencipe (1982), respectively.

Statistical analysis

The data is expressed as means±SEM. Comparisons among groups (n= 8) were performed by one-way analysis of variance (ANOVA) followed by the Tukey’s post-test, when indicated. Differences were considered statistically significant at P <0.05 (Zar, 1999).

RESULTS

Table I demonstrates a significant reduction (P<0.05) in RBCs count, Hct value and Hb content at 10 and 20 days post-exposure to 5Gy gamma-radiation. ß-glucan gavage treatment (50 mg / kg) of irradiated rats ameliorated the reduction of RBCs, Hct and Hb content as compared to irradiated group.

Single irradiation (5Gy) dose of gamma-rays induced a significant decrease in the total WBCs, absolute counts of lymphocytes and neutrophils. The animal group treated with oral ß-glucan post-irradiation and investigated after 10 and 20 days, showed significant increase in these parameters compared to the irradiated group (Table I).

Irradiation dose of 5Gy induced a significant (P<0.05) decrease in both bone marrow cell count and its viability percentage as well as spleenocytes viability. The male rats treated with ß-glucan post-irradiation and investigated after 10 and 20 days, revealed significant increase in that parameters compared to the irradiated group (Table I).
Table I shows significant increase in serum AsR and MDA level and a significant decrease in blood GSH level in the γ-irradiated groups compared with the control group. In the gavage administration of β-glucan, the inhibition of the serum AsR and MDA and enhancement of blood GSH level were significantly (p<0.05) imperative compared with the control and irradiated groups, which were more at 20 day than at 10 day post treatment.

Serum cholesterol and triglycerides levels in β-glucan treated animals were increased as compared with the control group, while γ-irradiation caused increase in their levels at 5Gy. Oral β-glucan treatment ameliorated the increase in cholesterol, triglycerides, which is more at 20 day than at 10 day post treatment (Table I).

**DISCUSSION**

The present results show that oral gavage treatment of β-glucan cause improvement in RBCs count, Hct value and Hb content as well as total WBCs, absolute lymphocytes and neutrophils counts. β-Glucan administration for 10 and 20 days also induced increase in BM cellularity and their viability percentage as well as spleen cell viability compared to the control group. This phenomenon was also observed by Zhao et al. (2005) and Yuan et al. (2009). They recorded an enhanced cellular immune response to glucan administration. Urao et al. (1999) suggested that the glucan administration allowed the beneficial microorganisms e.g., bifidobacterium to quickly reproduce in the animal intestine. These beneficial microorganisms can synthesize vitamin and amino acid, stimulate immunoglobulin activity and improve immune function. In addition, there are some reports on the immune-antioxidant activity relationship of glucan (Sener et al., 2007; Matheson and Caldwell, 2008), which may result in proliferation of bone marrow stem cells as indicated by increased in bone marrow cell count and it’s viability in the present study.

The current results show that γ-irradiation at dose of 5 Gy caused suppression in RBCs count, Hct value and Hb content as well as total WBCs, absolute lymphocytes and neutrophils counts. BM cellularity and their viability percentage as well as spleen cell viability was also decreased compared to the control groups. These results are in accordance with those of Salama (2009) and Hanafi et al. (2009). Wang et al. (2006) reported that exposure of C57BL/6 mice to 6.5Gy induced a quantitative and qualitative reduction of hemopoietic (HSC) stem cells, resulting from induction of senescence and impairment of HSC self-renewal via activation of the p53/p21 and p16/Rb pathways. This is complicated by thrombocytopenia and concomitant hemorrhages besides effects in adaptive immune system resulting from apoptosis of lymphocytes and deficient lymphopoiesis (Wilkins et al., 2002). Ionizing radiation is known to induce oxidative stress through generation of reactive oxygen (ROS) and nitrogen species (RNS) and resulting in imbalance of the pro-oxidant and antioxidant activities (Srinivasan et al., 2007; Tawfik and Salama, 2008).

β-Glucan administration post irradiation showed recovery of peripheral blood cell counts and Hct value and Hb content, facilitates recovery of decrease in BM count and its viability and spleen cell viability. This phenomenon was also observed by Pospilil et al. (1993) and Patchen et al. (1987) who concluded that the improvement effects of β-glucan when given to animals submitted to radiation, was not due only to hematopoietic regeneration, but also the capacity of this substance to inactivate free radicals. It is well documented that total body irradiation followed by β-glucan has been shown to raise the erythropoietic activity in both bone marrow and spleen (De Rooij et al., 2002).

Oliveira et al. (2009) indicated that β-glucan has a low potential in the prevention of teratogenesis. But, it appears to be effective in protecting against genetic damage, which increase host immune defense by activating complement system, enhancing macrophages and natural killer cell function through major β-glucan receptors and cytokine production.

Lipid peroxidation and ascorbyl free radicals mediated by oxygen free radicals is believed to be an important cause of destruction and damage to cell membranes and tissue injury as confirmed by the elevated AsR and MDA levels in the present study. On the other hand, γ-irradiation-induced MDA and
AsR increased levels were prevented by β-glucan and restored the GSH levels significantly, implicating an antioxidant effect of this glucose polymer. Sener et al. (2007) found that glucan administration could promote SOD and GSH-PX activity. Pyracanthula fortuneana Li polysaccharides (PFP) administration also increased the activity of SOD and GSH-PX and reduced the level of MDA. SOD and GSH-PX are major components of the antioxidant system in mammalian cells and work in concert to detoxify ROS, such as O₂⁻ and H₂O₂ (Yuan et al., 2010). β-Glucan of different origin has been demonstrated to be potent anti-oxidants, prevent damage by H₂O₂ and other reactive oxygen species (Angeli et al., 2006; Krizkova et al., 2003; Slamova et al., 2003). Free radicals directly have damaging effects on tissues or indirectly through activated neutrophils which secrete enzymes that liberate oxygen radicals (Vaziri, 2004). Also acute irradiation is characterized by inflammatory response mediated by cytokines that release large amounts of toxic oxidizing substances (Sener et al., 2005).

In the present study, serum cholesterol and triglycerides levels in β-glucan treated animals were decreased as compared with control group, while γ-irradiation at 5Gy caused increase in their levels; β-glucan gavage treatment however ameliorated the increase in cholesterol and triglycerides. Lipid metabolites were elevated in serum of irradiated animals which could be attributed to acceleration of other pathways of cholesterol formation in the liver and other tissues (Pulikova and Sedlakova, 1988). Free radicals destroy the cell membranes, enhance cholesterol release and increase the lipid peroxidation (Karbowink and Reiter, 2000).

Chen and Huang (2009) suggested that β-glucans sequester bile acids in the intestine, reducing their reabsorption and return to the liver. Reducing hepatic bile acid concentrations activate the enzyme CYP7A1, which converts cholesterol into bile acids. Drozdowski et al. (2010) concluded that the reduced intestinal fatty acid uptake after β-Glucan administration is associated with inhibition of genes regulating intestinal uptake and synthesis of lipids. Cholestyramine, like beta-glucans, also binds to the bile acids. Our result strongly indicate that β-glucans may up-regulate low-density lipoprotein receptor gene. These findings suggest that β-glucans may be used as alternative tool to treat irradiation induced oxidative stress. They enhance biological defense against oxidative stress by boosting immunological organs. Therapies directed against oxidative stress by food supplement treatments may be a potential strategy for irradiation treatment and chronic leukemia.

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